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                            Serial No. 09/775,386

### In the Claims

Claim 25 (currently amended) A method of operating absorbance-based chemical sensors to achieve calibration-free measurements, the method comprising the steps of:

a)      establishing wavelength accuracy to within about 2-3 nanometers;

b)      eliminating stray light at all wavelengths to about less than 0.1% incident light;

c)      preparing an analyte-selective reagent at a concentration;

d)      equilibrating the analyte-selective reagent to an analyte;

e)      taking an intensity reading of the equilibrated analyte-selective reagent and analyte at a first wavelength ($I_{\lambda 1}$) with a reagent-based optical chemical sensor, wherein the sensor has been modified to allow the renewal of an analyte-selective reagent, wherein the first wavelength corresponds to an un-reacted form of the analyte-selective reagent, and taking an intensity reading of the equilibrated analyte-selective reagent and analyte at a second wavelength ($I_{\lambda 2}$), wherein the second wavelength corresponds to a reacted form of the analyte-selective reagent;

f)      replacing the equilibrated analyte-selective reagent and analyte with a spectrophotometric blank solution;

g)      taking an intensity reading of the blank solution at the first wavelength ($I_{\lambda 10}$), and taking an intensity reading of the blank solution at the second wavelength ($I_{\lambda 20}$);

h)      calculating an absorbance ratio using the equation $A_R = A_{\lambda 1}/A_{\lambda 2}$, where $A_R$ is the absorbance ratio, $A_{\lambda 1}$ is absorbance at the first wavelength and $A_{\lambda 2}$ is absorbance at the second wavelength and, wherein $A_{\lambda 1}$ and $A_{\lambda 2}$ are determined by

$$\underline{A = - \log (I_\lambda / I_{\lambda 0})}$$

$$\cancel{A_\lambda = \log I_\lambda}$$

$$\cancel{I_{\lambda 0}} ; \text{ and}$$

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i)    calculating the sensor response with the molar absorptivities ($\epsilon$) of the reacted (a)

      form of the analyte-selective reagent and the un-reacted form (b) of the analyte-

      selective reagent using the equation

$$R = -\log\left(\frac{A_R - \epsilon_{\lambda 1a}/\epsilon_{\lambda 2a}}{\epsilon_{\lambda 1b}/\epsilon_{\lambda 2a} - A_R\epsilon_{\lambda 2b}/\epsilon_{\lambda 2a}}\right) = +pK_a - pH,$$

$$R = -\log\left(\frac{A_R - \epsilon_{\lambda 1a}/\epsilon_{\lambda 2a}}{\epsilon_{\lambda 1b}/\epsilon_{\lambda 2a} - A_R\epsilon_{\lambda 2b}/\epsilon_{\lambda 2a}}\right) = +pK_a - pH,$$

wherein when the analyte-selective reagent is prepared accurately and reproducibly at the

concentration sensor readings between sensors are calibration-free.